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Full Length Article

The evaluation of traditional and automatic Coulter method in estimation of haematological parameters in adult rats

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ABSTRACT

Haematology coulters are widely used in labs and hospitals in both diagnostic and treatment affairs. The aim of this study is to evaluate the performance of haematology coulter in comparison with the traditional manual method. Eighty blood samples obtained from adult albino rats were subjected to analysis in both methods. Results showed a significant ($p \leq 0.05$) elevation in values of red blood cells, haemoglobin and mean corpuscular haemoglobin obtained from coulter compared to those of manual method, whereas monocytes count increased in coulter compared to manual method. No significant difference was observed related to total white blood cells and its fractions. Indices of mean corpuscular volume, mean corpuscular haemoglobin concentration were not significant. In conclusion, the efficiency of coulter is better than manual method.

1. Introduction

Haematology analyzer is an automatic instrument programmed to perform certain functions to give an idea about the number of the blood cells through aspiration a blood sample to flow through an electric field allowing the detector to measure the number of the cells after diluting it with special solutions (Chaves, 2005). This method had proved its value when used clinically in hospitals instead of the traditional manual method that depends on the visual counts of the blood cells further more thousands of studies published in scientific journals throughout five decades ago under took the results of coulters (Marshall et al., 2008).

The principle of the automatic method was put by Coulter who had explained that the flow of the cells within charged fluid will generate pulses according to the size of these cells. Upon this principle we were able to manufacture machines that measure the number of the cells depending on its characteristics like size, surface area, and the granules within. Whereas haemoglobin estimated by reacting with a specific reagent (Rechner et al., 2002). The traditional method depends on manual counting of the RBCs under the microscope after dilution. As for the haemoglobin, it depends on transforming it to haematin and comparing its colour with standard colours, a process that takes time and efforts (Joe et al., 2011).

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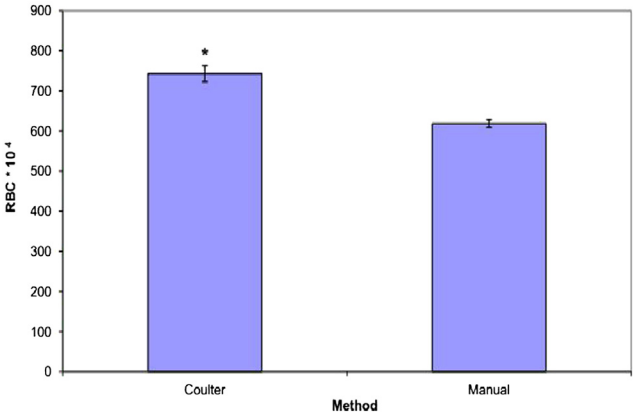

Fig. 1 – Comparing the number of RBCs between the automatic analyzer and manual method (Mean ± SR).

The current study aimed to evaluate the vet efficiency and of haematology counter machine compared to the manual method when used on blood samples taken from adult rats.

2. Materials and methods

This work was done in animal house of Veterinary Medicine College in University of Mousl, Iraq.

Study animals: 80 male albino rats, ageing 60 days, weight 160–190 g that had not been mixed with female rats. All were fed equally and put in cages 20 by 50 cm (5 rats/cage) in temperature of 22 ± 3 °C and humidity 42%. All the animals were feed on a diet mixed to meet the physiological requirement of rats.

Study design: Divided the animals into two groups for measuring and comparing the blood using manual and automatic method.

Blood sample collection: One ml of blood was collected from retro-orbital phlexus (Timm, 1979) in a capillary tube containing EDTA, in an average of 20 animals per day for 40 day.

Blood picture: Automatic method (automatic cell counter) Vet haematology analyzer was used (Abacus junior, Radim, Italy) after putting the samples on electric mixer. Each sample

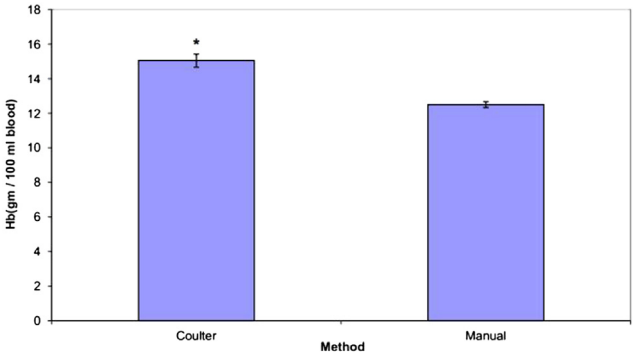

Fig. 2 – Comparing haemoglobin concentration between the automatic analyzer and manual method (Mean ± SR).

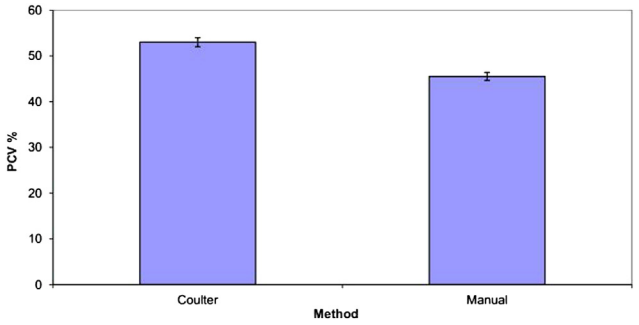

Fig. 3 – Comparing the packed cell volume between the automatic analyzer and manual method (Mean ± SR).

had been estimated in duplicate manner (mean of each duplicate was introduced to the statistic analysis).

Manual method: The number of the RBCs and white blood cells were counted in whole blood using a counting chamber for each after diluting the RBCs with Hayem's solution (Jain, 1986) and the WBCs with Turk's solution (Jain, 1986) then counting the number of the cells using light microscopy.

Differential count of WBCs was done after staining them with Leishman's stain and using Battement method for count 100 cells at the edge of counting chamber and then take the percentage for each type of white blood cells which include granulocyte (basophil, eosonophil and neutrophil) (Witko et al., 2000), monocyte and lymphocyte.

The haemoglobin concentration was measured using Shali method through converting the haemoglobin within RBCs to acid haematin after being treated with hydrochloric acid (Jain, 1986).

The percentage of packed cell volume was measured by taking a sample in a micro haematocrit tube that contains anti-coagulant and then centrifuged 1200 RPM for 10 min and then reading the results on a haematocrit reader. Then measurement the indexes Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) (Jain, 1986).

3. Statistical analysis

The statistical significance of differences observed between the coulter and manual method was analyzed using student

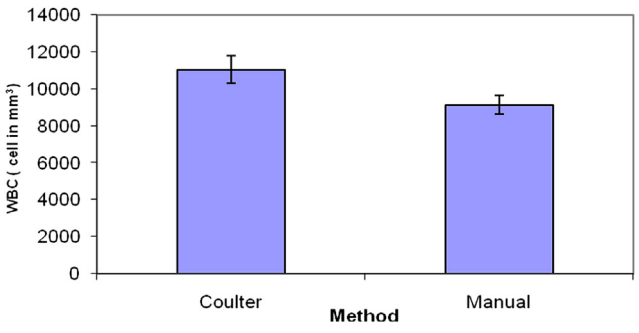

Fig. 4 – Comparing the number of WBCs between the automatic analyzer and manual method (Mean ± SE).

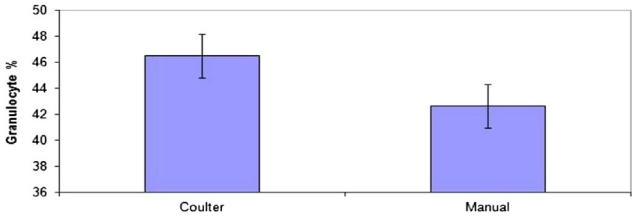

Fig. 5 – Comparing the percentage for number of granulocyte between the automatic analyzer and manual method (Mean ± SR).

independent (T-test) by mean of computer programme, statistical package for social sciences (Petric and Waston, 1999).

4. Results

The current study shows some inconsistency in the performance of the automatic analyzer as compared with the manual method. Fig. 1 shows statistically significant difference ($p \leq 0.05$) in the rate of RBCs between the two methods so the rates obtained through the automatic counter was 20% higher compared to the manual method. So was the case with the haemoglobin concentration as we found a significant difference ($p \leq 0.05$) between the results of the two methods with observing that mean of haemoglobin concentration is higher in coulter than manual method (Fig. 2). Whereas PCV values were obtained from coulter higher than manual method, however no significance was observed (Fig. 3).

There was increase but no statistically significant difference regarding the count of the WBCs (Fig. 4) or the number of the granulated cells (Fig. 5), lymphocytes (Fig. 6) and monocytes (Fig. 7). As for the rates (MCV, MCHC), they were similar between the two methods without significant differences (Figs. 8 & 9) while MCH had a significant increase in coutler method compared to manual (Fig. 10).

5. Discussion

The result obtained regarding the rate of the RBCs was statistically significant ($p \leq 0.05$) when measured with the

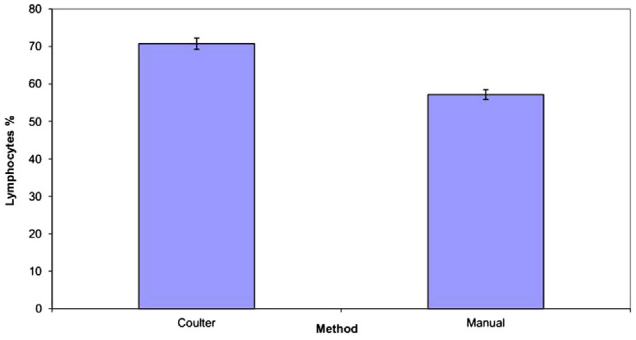

Fig. 6 – Comparing the percentage for number of lymphocyte between the automatic analyzer and manual method (Mean ± SR).

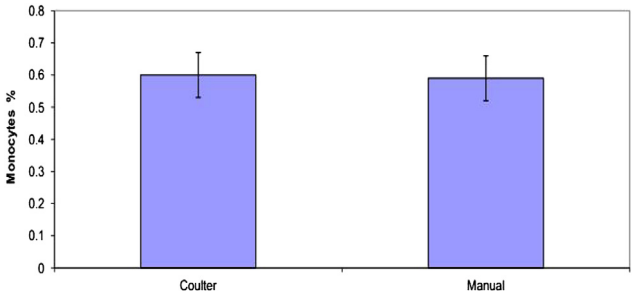

Fig. 7 – Comparing the percentage for number of monocyte between the automatic analyzer and manual method (Mean ± SR).

automatic method compared to the traditional manual method suggesting the passage of two or more RBC through the "flow cell", which is called "coincidence" causing to obtain a higher number than in the real sample (Lee et al., 2012; Jean et al., 2011). However, upon noticing the rates of haemoglobin concentration, it is also noticed that they are increased too in the samples when measured with the automatic method as

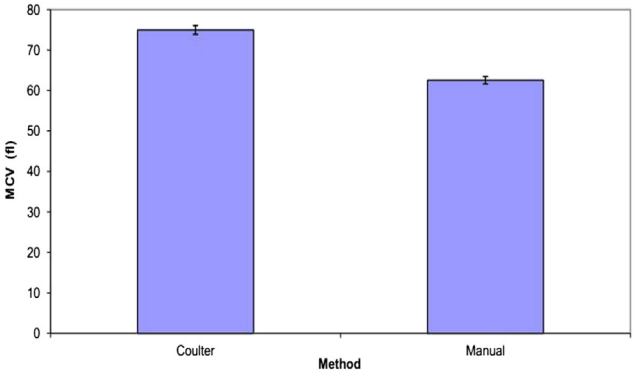

Fig. 8 – Comparing the mean corpuscular volume (MCV) between the automatic analyzer and manual method (Mean ± SR).

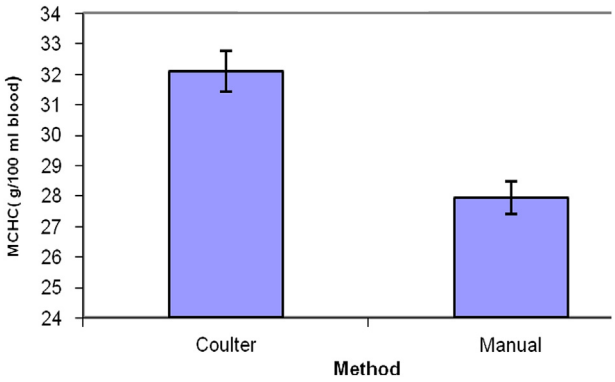

Fig. 9 – Comparing the mean corpuscular haemoglobin concentration between the automatic analyzer and manual method (Mean ± SE).

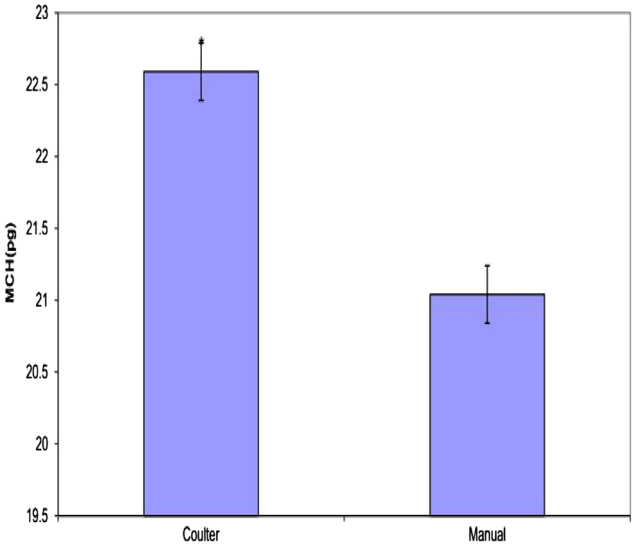

Fig. 10 – Comparing the mean corpuscular hemoglobin concentration between the automatic analyzer and manual method (Mean ± SR).

compared to the traditional manual method, which in concordance with what is mentioned above.

Regarding the number of the RBCs, it is known that the haemoglobin is present entirely within the RBC (Gyiton and Hell, 2007) and any method to measure it necessitate (to start with) the release of the haemoglobin from within the haemolysed RBC. Therefore, the result obtained with the automatic (Tan et al., 2011) method confirms the accuracy of the method regarding the occurrence of the "coincidence" which can explain the close results between the two methods.

The MCH increased significantly because it depends in RBCs count and haemoglobin concentration (Thrall, 2004; Bunn, 2011).

It is known that the traditional method of measuring RBC depends on the microscopical count while Hb depends on colour comparison by the naked eye, which carry inherent error in counting and dilution in contrast to the automatic method that dilute all the samples equally to be measured later by "light scattering" method (Sethi et al., 2010; Kwon et al., 2011; Kleine et al., 2012 Kwon et al., 2011; Hedley et al., 2012). However, it was noticed that the number of the mononuclear blood cells measured by the automatic method under microscopy was statistically more significant than that measured with the manual method; the automatic method is sensitive to the normal shape of the cells rather than other shapes of the mononuclear cells which may occur upon transformation during inflammation (Jo et al., 2011).

As for the white blood cells, there was no statistical difference when measured with the manual method compared to the automatic method.

It is worthy to mention that through the comparison of the results of current study with standard normal values (Mohammad, 2003) it is obvious that the coulter performance on the levels of both RBC count and Hb concentration was more precise than the manual method. It is concluded from the current study that using the automatic method to count the RBCs is just as reliable for diagnostic purposes as the manual method.

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